GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLE AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract

The development of nanotechnology interests the researchers for synthesis of nanoparticles with various bio-applications. Research on green production methods for metal oxide nanoparticles is growing with the objective to overcome the potential hazards of these chemicals for a safer environment. The present investigation was made to synthesis copper oxide nanoparticles by using medicinal plants Solanum nigrum and Solanum trilobatum. The bio-molecules present in the leaf extract react with copper sulphate, upon reduction at room temperature resulted in the formation of copper oxide nanoparticles. The biosynthesized copper oxide nanoparticles were characterized by using UV-Visible absorption spectroscopy, Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and Scanning electron microscopy (SEM) techniques. These analytical techniques clearly confirm the formation of copper oxide with monoclinic structure. The antimicrobial assays were demonstrated against some bacterial and fungal growth. The biologically synthesized CuO nanoparticles were found to be effective in controlling the growth of human pathogens Eschericia coli, Klebsiella and Staphylococcus aureus. The maximum zone of inhibition was found to be more in gram positive bacteria when compared to gram negative bacteria. Our method utilizes an economic, eco-friendly and biocompatible reducing agent for the synthesis of copper oxide nanoparticle.

Keywords : Solanum nigrum, Solanum trilobatum, UV-VIS, FT-IR, XRD, SEM, E.Coli, Klebsiella, S.aureus, Candida albicans.

1. INTRODUCTION

Nanotechnology is rapidly increasing field that making an impact on human life such as pharmaceutical, health, food, electronics, chemical industry, energy science, cosmetics, environmental science and space industries etc [1]. Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. Their unique size-dependent properties make these materials superior and indispensable in many areas of human activity. Nanoparticles possess unique physical and chemical properties due to their high surface area and nanoscale size [2]. There are many ways to synthesize nanoparticles such as sol gel method, chemical reaction, solid state reaction and co-precipitation. Compared to those methods, green synthesis method is one of the best method for the synthesis of nanoparticles in recent years. This method has several
advantages namely low cost, simple, use of less toxic materials, most important is eco-friendly. In this method, the plant extract has been used as reducing agent for the synthesis of copper nanoparticles [3].

Metal oxide nanoparticles (NPs) have been receiving considerable attention for their potential applications in nano sensors, nano devices, nano electronics, information storage, and catalysis [4]. Among various metal oxide NPs, copper oxide nanoparticles, due to their excellent physical and chemical properties and low cost of preparation, have been of great interest. Copper oxide nanoparticles have wide applications as heat transfer systems, antimicrobial materials, super strong materials, sensors and catalysts. Copper oxide nanoparticles are very reactive because of their high surface-to-volume ratio and can easily interact with other particles [5] and increase their antimicrobial efficiency.

In present study we prepare copper oxide nanoparticles from Solanum trilobatum, Solanum nigrum leaf extracts. Copper oxide nanoparticles act as antimicrobial agent in various fields. The copper oxide is highly toxic to microorganism such as bacteria (E-Coli, Klebsiella, Staphylococcus aureus, Candida albicans). The antimicrobial activities of synthesized copper oxide nanoparticles was tested and the formation of these nanoparticles was confirmed by UV-Visible, FT-IR and XRD spectroscopy techniques.

2. MATERIALS AND METHODS

2.1. MATERIALS

Copper sulphate (CuSO₄), Solanum nigrum (Black nightshade) leaves, Solanum trilobatum (purple fruited pea eggplant) leaves, de-ionized water. All glass wares were washed with sterile distilled water and dried.

2.2 METHODS

2.2.1. Preparation of the plant extract

Fresh leaves of Solanum Nigrum (Black Nightshade) and Solanum Trilobatum (purple fruited pea eggplant) were collected and 10 grams of collected green leaves were washed thoroughly with de-ionized water. 10 grams of the washed leaves were cut into fine pieces and crushed and boiled in 100 ml of de-ionized water, for half an hour. The aqueous extract thus obtained was filtered through filter paper to obtain a clear extract. The extract was collected in clean and dried 100 ml beaker. Then the filtrates were collected and refrigerated for further experiments.

2.2.2. Synthesis of Copper Oxide Nanoparticle

For the preparation of CuO nanoparticle, 15 ml of 0.01 M of CuSO₄.5H₂O was added to 5 ml of the plant extracts e.g. Solanum nigrum (Black nightshade leaves) and Solanum trilobatum (Purple fruited pea eggplant) in 3:1 ratio and it was kept in magnetic stirrer for an hour. The colour change of the solution from brown to green was observed in Solanum nigrum. The colour change of the solution from brown to pale green was observed in Solanum trilobatum. The change of the colour takes place within few minutes and the
precipitate was formed. This solution was centrifuged and the precipitate was separated. Then it was dried to get powder form.

2.3. Characterization of CuO Nanoparticle

UV – Visible Spectrophotometer was used to record the localized surface plasmon resonance of copper oxide nanoparticles at 200 – 380 nm. FTIR spectrum was recorded in mid IR region in the range of 400 – 4000 wavenumber (cm\(^{-1}\)). The structure and size of the nanoparticles was obtained from X-ray diffraction (XRD) technique. Morphology were examined using Scanning electronic Microscopy (SEM).

2.4 Antimicrobial activity

Antimicrobial activity of metal oxide nanoparticles depends on size, stability and concentration in the growth medium. Antimicrobial activity results that CuO Nanoparticles resistant to both bacteria and fungi. Copper oxide nanoparticles have been known to have inhibitory and bactericidal effects and thus we extend its application as an antibacterial agent. The antimicrobial assay was done on human Klebsiella, E.Coli, Staphylococcus aureus and Candida albicans by the Kirby Bauer method.

3. RESULTS AND DISCUSSION

3.1. UV-Visible spectroscopy

UV-Visible spectroscopy analysis showed that the wave length of Copper Oxide nanoparticle synthesized using Solanum Nigrum, Solanum Trilobatum extracts centered at 200-380 nm due to the excitation of surface plasmon vibrations in the CuO nanoparticles. The figures 1 and 2 represent the UV-Visible spectrum of CuO nanoparticles.

![UV-Visible spectrum of CuO nanoparticles from Solanum Nigrum](image-url)
From the UV-Visible spectra, it was observed that absorption peak was detected at 320 nm, 212 nm as the concentration of Solanum nigrum, Solanum trilobatum leaf extract was increases respectively. The appearance peak indicate the formation of CuO nanoparticles.

### 3.2. FT-IR SPECTRAL STUDY

Infrared spectroscopy is an important record which gives sufficient information about the structure of the compounds. The most important region for an organic chemist 2.5µ to 15µ. The region from 0.8µ to 2.5µ is called near infrared region and 15µ to 200µ is called Far IR region.
The presence of Copper oxide nanoparticles was identified by the strong absorption band at 617 cm$^{-1}$ using Solanum Nigrum leaf extract.

![FT-IR Spectrum for CuO Nanoparticles from Solanum Trilobatum](image)

**Figure 4.** FT-IR Spectrum for CuO Nanoparticles from Solanum Trilobatum

The presence of Copper oxide nanoparticles was identified by the strong absorption band at 621 cm$^{-1}$ using Solanum Trilobatum leaf extract.

The FT-IR spectra of metal sample show specific stretching vibrations for the different structural forms of metal. The specific metal oxide and their IR vibrational frequencies are shown. The stretching frequencies are observed at 500-4000 cm$^{-1}$. It is confirmed that the obtained nano metal oxide was CuO.

### 3.3 X-RAY DIFFRACTION STUDIES

The XRD spectrum is recorded by X-Ray diffractometer with Miniflex 600 Desktop [first support]. The average particle size is determined using **Debye-Scherrer’s equation** applied to major, peaks corresponding to maximum intensity in the XRD pattern of the samples.

The size of the synthesized CuO Nanoparticles were calculated from powder XRD Pattern using Scherrer’s formula.

\[
D = \frac{k \lambda}{\beta \cos \theta}
\]
The size of the CuO nanoparticles thus estimated was found to be 4.4490 nm for Solanum nigrum.

The size of the CuO nanoparticles thus estimated was found to be 5.0679 nm for Solanum trilobatum. The observed “2θ” values come in good agreement with standard “2θ” values. This XRD value confirms that the synthesized particles were nanometric in the size.

3.4 SEM ANALYSIS

The SEM is recorded by JOEL Model 6390 computer-controlled microscope. The image obtained by SEM of the samples for CuO shows solid block like nanoparticles. The CuO Nanoparticles have been distributed well within the range of ~100 nm which is the favourable for some other purpose. It was shown that the samples of CuO synthesized are having particle size in the Nano scale.
3.5 ANTIMICROBIAL ACTIVITY

The antimicrobial activity of CuO nanoparticles were investigated for both bacteria (E.coli, Klebsiella, Staph aureus) and fungi (candida albicans) by kirby-bauer method. The antimicrobial activity of CuO nanoparticles against E.coli, Klebsiella, Staph aureus and thus antifungus activity of CuO nanoparticles against candida albicans are shown in Fig. 9.
Fig 9. Antimicrobial activity of Solanum nigrum, Solanum trilobatum

Table 1: Antimicrobial Activity For Biosynthesis Of Copper Oxide Nanoparticles

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>SOLANUM NIGRUM</th>
<th>SOLANUM TRILOBATUM</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>8 mm</td>
<td>8 mm</td>
<td>20 mm</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>10 mm</td>
<td>11 mm</td>
<td>25 mm</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>11 mm</td>
<td>9 mm</td>
<td>17 mm</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10 mm</td>
<td>11 mm</td>
<td>25 mm</td>
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</tbody>
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The maximum zone of inhibition was found to be more in gram positive bacteria when compared to gram negative bacteria. The antimicrobial activity results that CuO Nanoparticles resistant to both bacteria and fungi.

5. CONCLUSION

The copper oxide nanoparticles were successfully synthesized by using novel Solanum Nigrum and Solanum Trilobatum leaf extracts, as first time for the antimicrobial study, which provides cost effective, easy and proficient way for synthesis of copper oxide nanoparticles. The sample obtained by the green synthesis was characterized by UV-Visible, FT-IR, XRD and SEM instrumental methods. The UV-Visible spectra gives the peak at 200-380 nm, it shows the presence of copper oxide peak. The FT-IR analysis of the spectra shows broad band between 500-4000 cm\(^{-1}\) with shoulder shape, characteristics of CuO band. From the XRD results the size of CuO Nanoparticles were calculated to be less than 10 nm. The CuO Nanoparticles have been distributed well within the range of \(\approx 100\) nm, so it can be used to some other purpose. The image obtained by SEM of samples CuO shows solid block shape of nanoparticles. Copper oxide nanoparticles were effectively utilized for the antimicrobial activity study. The maximum zone of inhibition was found to be more in gram positive bacteria when compared to gram negative bacteria. The antimicrobial activity results that CuO Nanoparticles resistant to both bacteria and fungi. The Solanum Nigrum, Solanum Trilobatum leaf extracts are effectively utilized for the production of copper oxide nanoparticles with economically for many pharmaceutical applications.

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